Polyacrylamide Protein Gel Electrophoresis (PAGE)
Standard Operating Procedure

Purpose:

The goal of protein electrophoresis is to detect proteins by separating them by their molecular weight.

Materials and Equipment:

Casting the Gel

- 2 glass plates
- Gel Spacer
- Casting Support
- 40% Acrylamide
- Stacking Buffer
- Resolving Buffer
- d_2H_2O
- 10% Ammonium persulfate (APS) Solution
- Tetramethyleethylenediamine (TEMED)
- Butanol
- Well Comb

Sample Preparation

- SDS Sample Buffer
- Dithiothritol (DTT)

Running the Gel

Vertical Electrophoresis Cell

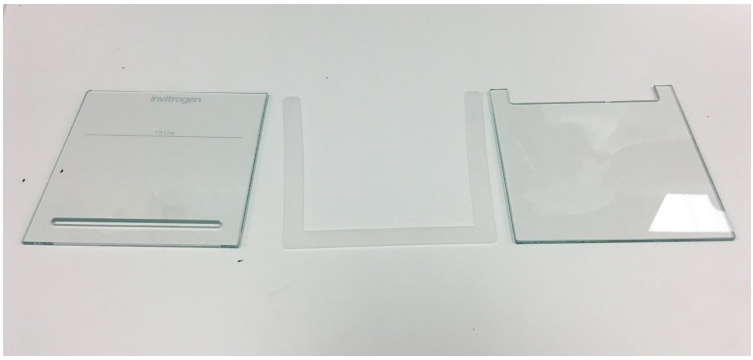
- Electrophoresis Housing Unit
- Electrophoresis Gel Cast Support with Electrodes
- Lid
- Tris-Glycine Running Buffer (1x concentration)
- Power Supply (PowerPac Basic Power Supply)

Procedure:

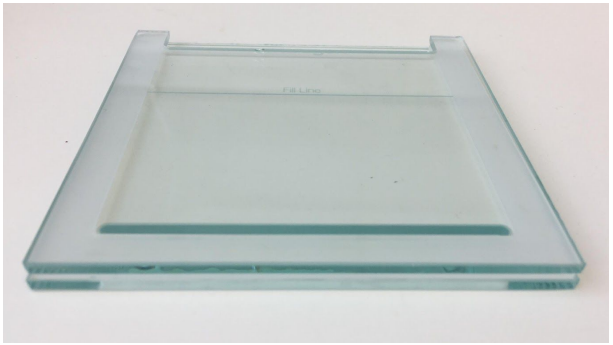
Casting the Gel

1. Setting up the vertical loading tray

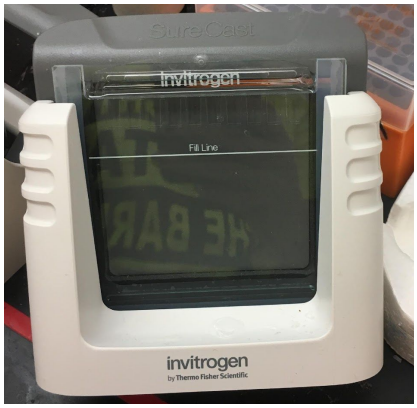
a b c



The gel spacer(b) is placed on top of the Invitrogen Labeled Glass Plate(a) so that the spacer aligns with the edges like a border. The final glass plate(c) is placed on top of the gel spacer keeping the groove at the top.



Finally, the recently assembled Glass Casting Apparatus is placed upright into the Gel Casting Apparatus Support. The Invitrogen Plate with the open groove at the bottom is against the back. The blank plate with the groove at the top is held against the front. The spacer stays between the two plates. The gray tab is pulled forward against the back of the glass plates to lock them in place. Placing this properly helps when it comes time to pour in the gel.



2. Making gel

Protein gels are made with two different types of gels, resolving gel and stacking gel. The stacking gel is the top gel layer and the resolving gel is the bottom gel layer.* ***Acrylamide is used in both gel layers and is considered a health hazard because it is a carcinogen, mutagen, reproductive toxin, etc. Therefore, it should be handled in a fume hood. Take extra precautions to avoid the acrylamide making contact with skin.*

- The resolving gel is made up of the following in a conical tube. Percent acrylamide can be changed. For other concentrations, check the SureCast Invitrogen Packet.

For a 10% resolving gel solution add…

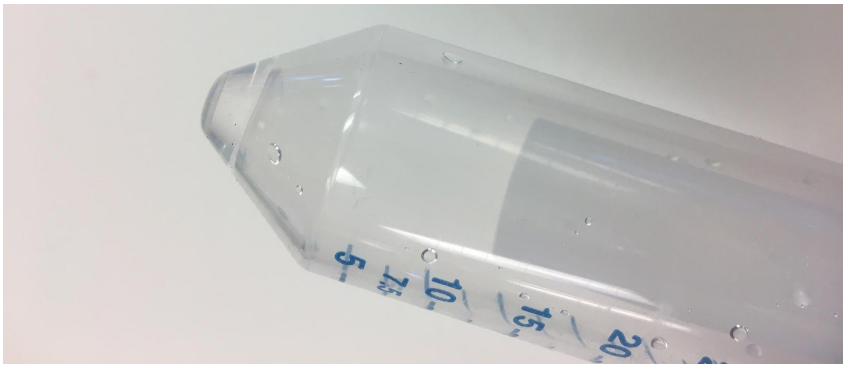
- 2.0 mL (40%) acrylamide
- 2.0 mL resolving buffer
- 3.9 mL distilled water
- 80 µL (10%) APS

**The following 4 steps should be completed as quickly as possible because the gel will start to polymerize soon after the TEMED has been added.*

- Quickly add 8 µL of TEMED
- Briefly agitate lightly to mix (avoid bubbles if possible)
- Pour the solution in between the two glass cast plates to the fill line in the preset loading tray (not all of the resolving gel will be used)

Pipette butanol over the top in between the two glass cast plates until it fills the rest of the space to the top. **This is done to protect the gel from reacting with oxygen and to help the gel settle evenly which is critical for getting your proteins to enter the resolving gel evenly. Any imperfections will cause certain lanes to run faster or slower due to the change in acrylamide percentage from the resolving to the stacking gel.*

- An indicator for solidification of the resolving gel is when the leftover material is firm in the bottom of the 50 mL conical tube.



**The conical tube may be cleaned out and reused for the stacking gel*

- Absorb the butanol away from the resolving gel with filter paper (avoid touching the paper to the gel). Excess butanol will prevent the polymerization between the two gels and will ultimately decrease the quality of the gel.

- The stacking gel is made of the following in a conical tube
    - 300 µL (40%) acrylamide
    - 750 µL stacking buffer
    - 1.92 mL distilled water
    - 30 µL (10%) APS

**The next 4 steps should be completed as quickly as possible similar to the resolving gel*

- Quickly mix in 3 µL TEMED
- Briefly agitate lightly to mix (avoid bubbles if possible)
- Pour on top of the resolving gel, to the top of the divot (overflow out of the cast is okay/encouraged, but still be careful)
- Place comb snuggly into the gel with the flat, smooth side facing the back, pressed against the glass

**Comparable to the resolving gel, the stacking gel is set when the leftover material is firm in the bottom of the 50 mL conical tube*

Sample preparation

**to be done in a sterile environment*

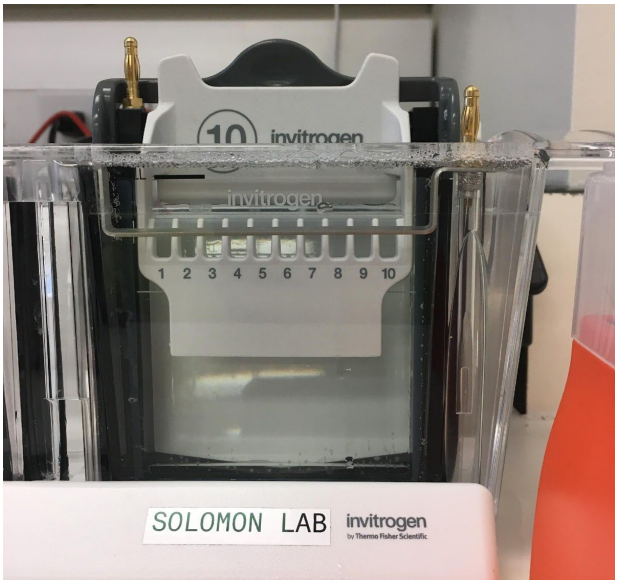
- For each of the samples, add the following into a 1mL conical centrifuge tube
    - 20 µL SDS Sample Buffer
    - 2 µL DTT
    - 18 µL of 10-20 µg Desired Protein Sample (if overloaded or highly concentrated, the gel
- Heat at 95C for 2 min

Running the gel

- Place the Electrophoresis Gel Cast Support with Electrodes into the Electrophoresis Housing Unit (make sure the electrodes align with the electrode insertion points on the Housing Lid)
- Partly fill the Electrophoresis Housing Unit with Tris-Glycine Buffer
- Slowly remove comb from the casted protein gel vertically (avoid pulling at an angle because this could lead to the destruction of the wells set up by the comb)
- Place the glass plates containing the gel inside the housing unit full of the running buffer. Use the grey/black locking support with the electrode on it to lock the glass plates in place by pulling the grey handle towards the glass plate. This locks the plates with the gel in place towards the front of the housing unit.
- Fill the rest of the unit with Tris-Glycine Buffer until it slightly overflows into the sides

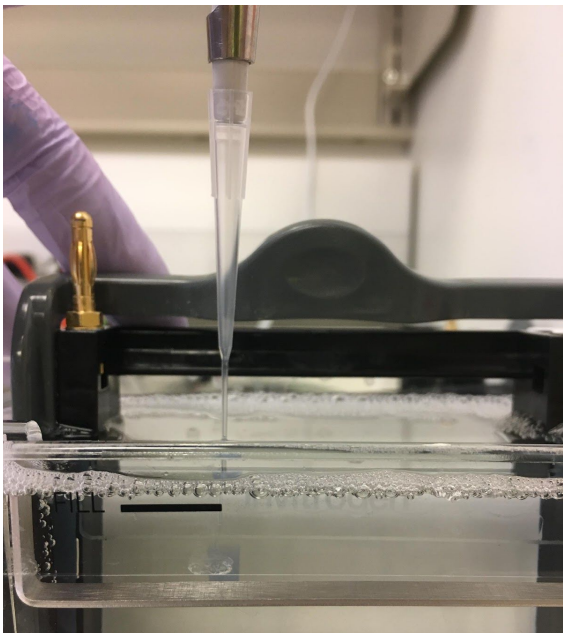
Loading the gel

**If desired, set the Well Guide so that it sits on top of the glass plates and the well guides lay across the front. This can be helpful if it is hard to see the wells through the buffer.*



- Use Protein Gel Loading tips and load each sample into its own well (each well can hold up to 40 µL, unless larger wells are used in which case it could hold more)

**include a well with 5.5 µL of PageRuler Plus Protein Ladder to accurately determine the size of the proteins in each of the bands when imaged later on*



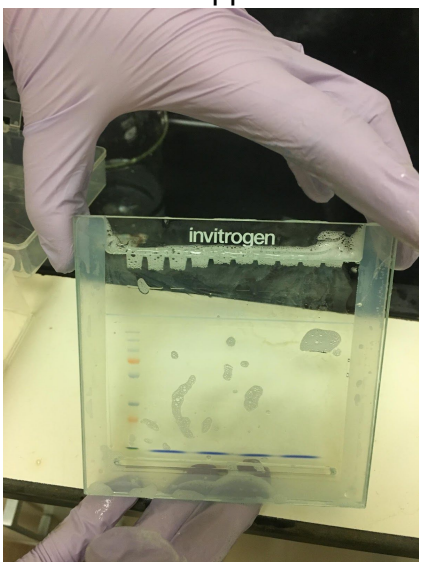
- After each sample is loaded, place the lid on top of the housing unit and make sure that the electrodes align with the wires to the power source

- Connect the wires to the power source
- Turn on the power source
- Set the timer to approximately 100 minutes and the Voltage to 55V and press the run button
- Once the samples have passed through the stacking gel, increase the voltage to 110V or higher.

**110V is a relatively lower voltage. It's not uncommon to use voltages at 120 or higher. However, there is a greater danger of the gel melting or warping at these temperatures. This causes smiling in the gel (where the bands angle up on the outermost lanes). Constant current (Amps) may also be used, but could run the proteins through the gel faster and could heat up the running buffer. This can cause messy results or warped edges on the gel.*

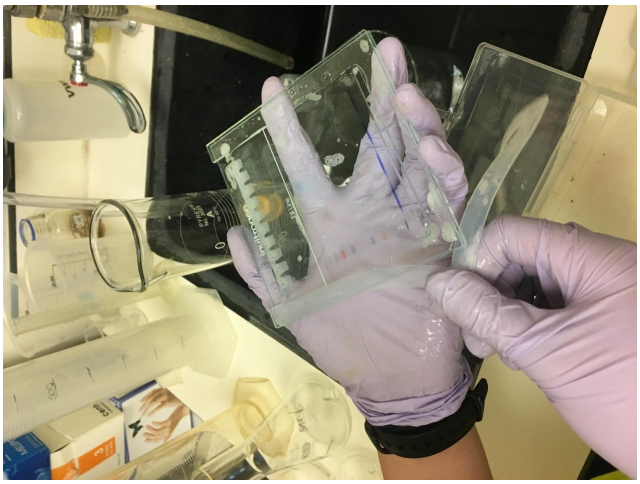
- Keep the samples running through the gel until the samples have reached the area just above the groove that exists at the bottom of the plate in the back of the Casting Apparatus

- Turn off the Power source
- Remove the lid
- Remove the Casting Apparatus from the Housing Unit by unlocking the Cast Support

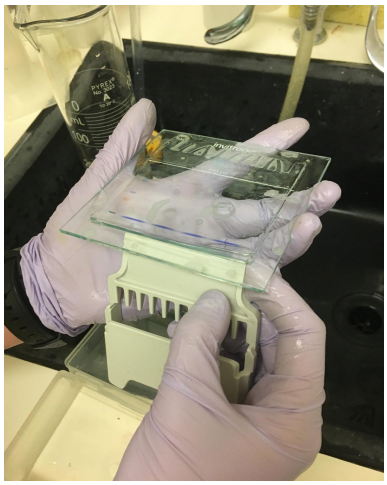


****Be extremely careful in the following steps because the gel is very thin and extremely susceptible to rips and tears***

- Use the edge of the Well Guide to pry away the Gel Spacer and lift the top plate from the bottom plate with the bottom groove



- Use the Well guide to push the gel up through the groove



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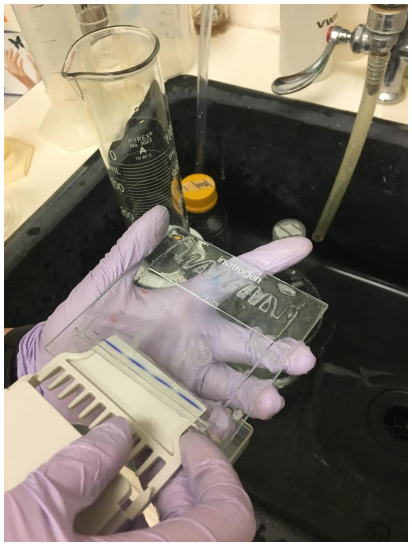
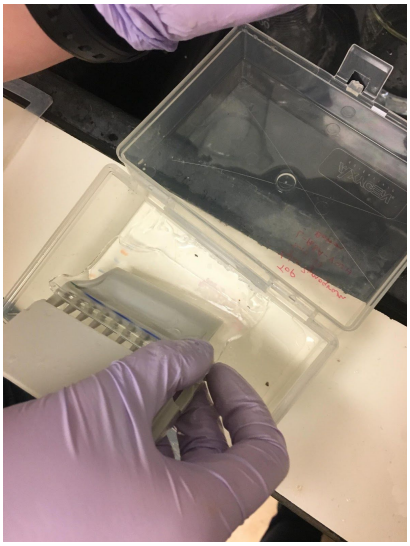
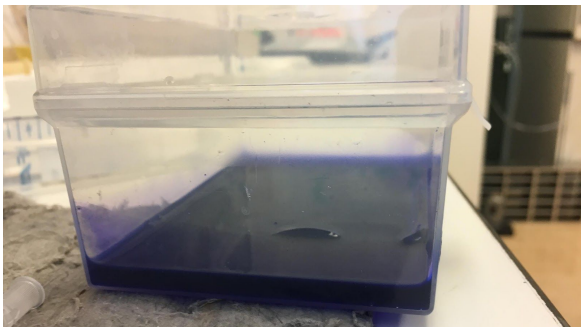
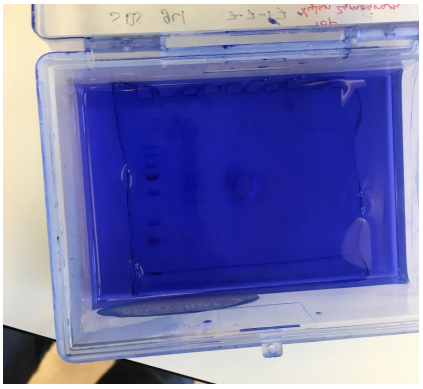
- Carefully lift the gel and place into an empty box





1. Staining the gel
    - Fill the box with the protein gel with Commassie G Stain

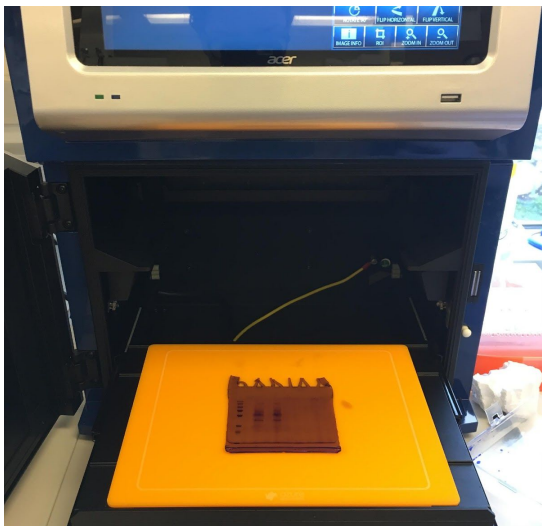



- Lightly shake box on a shaker plate at room temperature for 45 minutes

1. Destaining the gel
   - Slowly drain the box with the gel and stain (careful not to pour out or rip the gel)

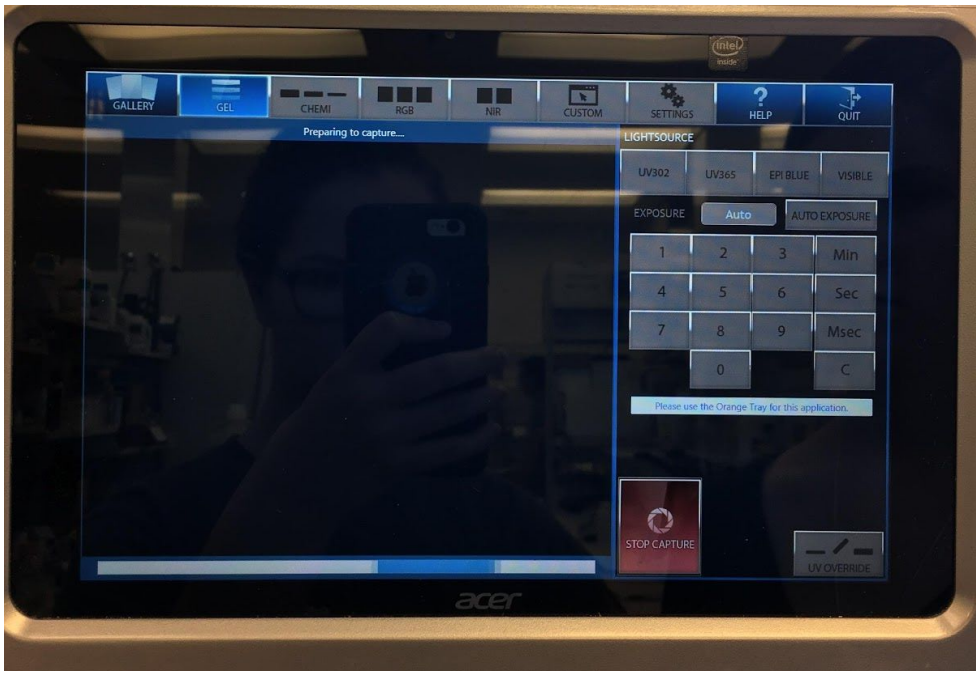
The stain should <u>NOT</u> be poured down the drain. It can be put in a container to be reused, or in a separate Coomassie waste container.

   - Refill the box with Destaining Buffer (10% glacial acetic acid and10% methanol, when making destaining buffer, add water first) and place on shaker plate at room temperature for ~2hrs.
   - Repeat last step until the bands appear against a relatively clear background (Usually fresh destain must be added a couple times to remove enough stain)
   - Leave gel in destaining buffer on shaker plate shaking overnight
2. Imaging the gel
   - Turn on the Imager and open the imaging program
   - Select Gel
   - Remove the gel from the shaker plate
   - Carefully remove the gel from the box with destaining buffer and place it on the orange plate inside the imager

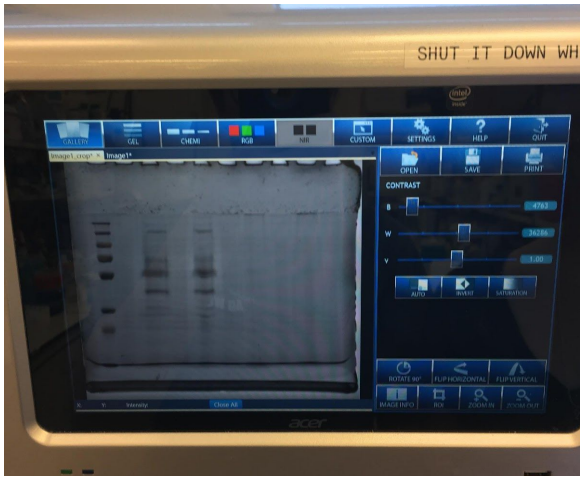


   - Close the door to the imager with the gel inside

- Select Visible for the light setting and Auto Exposure for the time setting
- Select Capture



- Wait for the image to appear on the screen
- Edit the picture with the settings to the right



- Save and print the image

Troubleshooting (tips/ how to fix issues):

- Gel not solidifying (either resolving gel or stacking gel):
  - There could be too little APS or TEMED
  - Incorrect concentration or amount of acrylamide
  - Old APS Solution
- Bands curve within gel lane:
  - Overloaded protein

    - Overheating the gel, lower voltage/current
    - Incorrect running conditions
- Vertical streaking:
    - Overloaded samples
    - Sample precipitation
    - Samples contain other materials (try a buffer exchange so that some of the other compounds can be removed)
- For further troubleshooting visit

http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf

References:

SureCast Invitrogen Packet
http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf